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EFFECT OF CORIANDER (*Coriandrum sativum* L.) ESSENTIAL OIL CULTURE ON SOIL BIOGENICITY AND DETERMINATION OF ITS ANTIMICROBIAL ACTIVITY AGAINST *Escherichia coli*

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Abstract

Soil microbiological and agrochemical indicators were analyzed during biological cultivation of coriander, in greenhouse conditions, as main indicators of good plant development, studied for antimicrobial activity against E. coli, by testing different variants of plant extracts (decoction, tincture, medicinal wine, medicinal vinegar, medicinal oil). The results of the agrochemical analysis show that coriander does not have a major impact on the dynamics of macronutrients in the soil, while the biogenicity and activity of enzymes cellulase and catalase increased in soils with coriander culture compared to the no-vegetation control. Positive antimicrobial activity against the pathogenic microorganism E. coli was reported for all variants of coriander extracts, differing for individual parts of the plant and individual variant extracts. Root and whole plant extracts showed higher antimicrobial activity compared to leaf and stem extracts. The strongest antimicrobial activity of the plant extracts was found in the medicated oil and medicated vinegar variants and the weakest in the `decoction` variants. The choice of solvent and exposure time likely influence the diameter of the retention zone.

Key words*: coriander, soil microorganisms, cellulase, catalase, antimicrobial activity.*

INTRODUCTION

The study of essential oil crops as biocides possessing antimicrobial activity is essential to determine their qualities as biological agents against pathogenic microorganisms. The term "biocide" is a uniting term for the antibacterial action of various substances (Russell, 2002). By their functional essence, these chemical substances attack and inactivate bacteria, exerting a toxic effect on their cells. Most often, the mechanisms include: disruption of bacterial cell homeostasis, lysis followed by the leakage of the internal contents of the cell, inhibition of the catalytic function of bacterial enzymes, disruption of electron transport and oxidation processes, negative interaction with macromolecules and biosynthetic processes of bacteria (McDonnell & Russell, 1999). Coriander (*Coriandrum sativum* L.) is a well-known herb of the Apiaceae family, widely used as a spice in the food industry, as well as for its medicinal properties in pharmacy. Coriander seed oil is the second most important essential oil in the world, exhibiting antimicrobial activity against Gram-positive and Gram-negative bacteria, some yeasts, dermatophytes and filamentous fungi (Silva & Domingues, 2017). Coriander essential oil showed the strongest antibacterial activity against *Bacillus subtilis*, followed by *Stenotropomonas maltophilia* and *Penicillium expansum* (Kačániová et al., 2020). Coriander seed extract has the highest rate of growth reduction of several pathogenic or alteration microorganisms (*Salmonella typhi*, *Staphylococcus aureus*, *Candida tropicals*, *Mucor* sp., *Emericella nidulans*), while *Aspergillus flavus* has the highest resistance against coriander extracts (Amin et al., 2021). The antimicrobial mechanisms found in these essential oils have been explained on the basis of their content in natural compounds such as carvacrol, thymol, p-cymene and c-terpinene, among others (Amin et al., 2021). According to a study by Saygi et al. (2021) in terms of antimicrobial activity, the Gram-negative bacterium *E. coli* was more sensitive to coriander oil than the gram-positive bacterium *S. aureus.*

Soil biogenicity depends on a number of factors - soil temperature and humidity, soil mechanical composition, soil pH, nutrient supply, type of

vegetation, methods of soil treatment and fertilization, and other factors (Malcheva et al., 2018). A complex of factors (physico-chemical, chemical) affects the development of microorganisms and their activity, including the type of vegetation - differences are found in terms of the quantity, quality composition and activity of soil microorganisms when fertilizing with the same fertilizer products, but applied at different vegetation (Malcheva et al., 2018; 2019). Soil microorganisms and the enzymes produced by them are sensitive indicators of ongoing changes, including when growing essential oil crops (Malcheva et al., 2019). When studying the influence of fertilizers on the quantity and activity of soil microorganisms, Plamenov et al. (2016) found that, for equal treatments, soil biogenicity (mainly determined by bacteria, less actinomycetes and molds) was higher in the essential oil crop canola compared to wheat. Among different studied indexes the microbiological and enzyme activity are suggested by number of authors as universal indexes of the soil fertility and pollution (including contamination with pathogenic microorganisms) (Malcheva et al., 2021; Malcheva et al., 2022, Dilly et al. , 2003; Nannipieri et al., 2000; Li et al., 2008; Perucci, 1992; Pascual et al., 1999; García-Gil et al., 2000; Ros et al., 2003; Crecchio et al., 2004 ; Bastida et al., 2008; Marcote et al., 2001; Malcheva, 2014a; 2014b). The biochemical indices are most sensitive towards the changes in the soil properties and their measurement are used largely as indicators for the management effect over the condition of the soil and agricultural productivity (Bandick and Dick, 1999; Nayak et al., 2007).

Essential oils are used as a source of carbon and energy by quite ubiquitous soil microorganisms and provide evidence that they will not accumulate in soil if environmental conditions favor the growth of these microorganisms (Vokou and Liotiri, 1999). In a study of the herbicidal potential of essential oils, Jouini et al. (2020) found that soil microorganisms, after a transient shock period induced by the addition of essential oils, recovered their original function and biomass.

Plants produce secondary metabolites that can inhibit bacteria, fungi, viruses and pests. There is a general consensus that secondary metabolites in plant extracts can inhibit Gram-positive bacteria more than Gram-negative bacteria (Ait-Ouazzou et al., 2012; Chanda et al., 2011), i.e. Gram-positive bacteria are more susceptible to plant extracts (Rakholiya et al., 2013). This difference is simply a consequence of the difference in cell wall structure between these major classes of bacteria. The cell wall of Gram-negative bacteria is surrounded by an additional lipopolysaccharide membrane that provides a hydrophilic surface and functions as a permeability barrier for many plant extracts. However, this is not always true, as some plant extracts inhibit Gram-negative bacteria more than Gram-positive bacteria.

Over 1,340 plants have been identified with antimicrobial activity, and over 30,000 antimicrobial compounds have been isolated from plants (Tajkarimi et al., 2010; Vaou et al., 2021).. Medicinal plants and their natural products remain largely untapped as sources of antibacterial compounds. Eloff (2004) gives certain criteria for the efficacy of phytoproducts as follows: an extract or fraction is considered to have significant antibacterial activity if the minimum inhibitory concentration (MIC) against the given pathogenic microorganism is equal to or less than 100 µg /mL, and Kuete and Efferth (2010) defined compounds with significant antibacterial activity as those with an MIC equal to or less than 10 µg/mL. Gibbons (2004) in his study defined essential oils as having significant antibacterial activity if the MIC was equal to or less than 5 µL/mL. Since the density of essential oils is lower but close to 1 g/mL, the MIC value of essential oils = < 5 µg/mL is considered to be relevant and can serve as a reference limit. Following these criteria, 50 essential oils have been reported to possess high antibacterial activity against at least one bacterial species. Such observations confirm that plants and their natural products represent promising sources of antibacterial agents and that continued research is needed in this direction.

The purpose of the study is, on the one hand, to determine the influence of the coriander essential oil crop on the soil microflora, and on the other hand, to analyze the antimicrobial activity of the plant against the pathogenic microbial species *E. coli*.

MATERIALS AND METHODS

The experiment was carried out under controlled conditions in the greenhouse of the educational and experimental field of the Department of Plant Breeding at the Technical University - Varna.

Before planting the experiment, during the growing season and after harvesting the crop, soil samples were taken, for each of the options, to determine:

- The content of ammonium nitrogen (NH4–N) and nitrate nitrogen (NO3–N) was determined spectrophotometrically.
- The content of phosphorus and potassium was determined by the Egner-Riem double-lactate method.
- Soil pH (ISO 10390).

For the microbiological analysis, the method of dilution and triplicate inoculation of solid nutrient media was used with subsequent counting of colony-forming units (CFU) in 1 g abs. dry soil (Mishustin & Emtsev, 1989; Malcheva & Naskova, 2018; Nustorova & Malcheva, 2020). Systematic and physiological groups of aerobic microbes - bacilli and non-spore-forming bacteria (on nutrient agar), micromycetes (mold fungi) - on Chapek-Dox agar, actinomycetes and bacteria assimilating mineral nitrogen (on Actinomycetes isolation agar) were determined. The general microflora was determined. The mineralization coefficient was calculated according to the formula: bacteria assimilating mineral nitrogen / (non-spore-forming bacteria+bacilli) (Mishustin and Runov, 1957; Malcheva and Naskova, 2018).

To isolate *E. coli*, a solid culture was made on Endo agar. Typical *E. coli* colonies on Endo agar are dark red with a metallic sheen (Malcheva and Naskova, 2020). Certified reference material was used: *E. coli* WDCM 0090 VT000904.

Agar diffusion method is used to determine antimicrobial activity (Nustorova and Malcheva, 2020). The volume of inoculated extract in each well was 60 µl.

The catalase activity of soil microorganisms was determined by the manganometric method (Khaziev, 1976).

In the laboratory experiment to determine the cellulose-decomposing activity, soil with a thickness of about 7 mm was poured into a petri dish with a diameter of 10 cm, maintaining 60% PPV /maximum field moisture content/. In each Petri dish, 3 strips of sterile filter paper measuring 10/50 mm are placed on the soil and cultivated at 25°C. During 10 days, the area of the degraded cellulose is recorded with a standard mesh. Average values from the three bands are calculated.

The following variants of coriander extracts were prepared (Table 1).

Table 1. Extract variants

Variant	Method of preparation of the extract
Decoct	A decoction (potion) is the liquid obtained by boiling the chopped plant product with the necessary solvent, usually water. Recommended for roots, flowers, leaves, twigs, fruits. The extractive solution is filtered while hot.
Tincture	Therapeutic substances are extracted from the chopped herbs by soaking with ethyl alcohol at a concentration of 30%, usually for a time varying between 8-10 days. The operation is carried out in well-closed glass containers. Shaking, for good extraction, is necessary throughout the extraction period.
Medicinal wine	The extraction is carried out in a weak hydroalcoholic environment, at a slightly acidic pH. For preparation, pre-crushed herbs are soaked for 7-10 days in wine (of good quality and well stabilized), after which the preparation is filtered.
Medicinal vinegar	It is obtained by extracting the active substances from herbal drugs with wine vinegar. For preparation, previously crushed herbs are soaked for 7-10 days in vinegar (good quality), after which the preparation is filtered.
Medicinal oil	It is a form of soaking the herbs in olive oil. The duration of soaking is 4-6 weeks. Store in tightly closed glass bottles, in a dark and cool place.

Coriander extracts are available in the following variants: roots, stems, leaves, whole plant.

RESULTS AND DISCUSSIONS

An agrochemical analysis of soil samples was peformed before sowing coriander and at the end of the vegetation of the plant species (Table 2).

Table 2. Agrochemical analysis

Variant	pH	Macronutrients			
		NH_4 mg/kg	NO_3 mg/kg	P_2O_5 mg/100 g	K_2O mg/100 g
Control (no vegetation)	7.26	4.03	8.24	17.1	17.7
Coriandrum sativum L.	7.24	3.97	5.99	16.2	15.4

Although after sowing the coriander, the values of the available forms of N, P and K decrease comparing with the limit values for the stocking of the soil with available nitrogen compounds, mobile phosphates and available potassium, it can be concluded that the soil is poorly stocked with nitrogen. In revanche, it has a good degree of storage in terms of phosphorus and potassium, and the soil reaction is slightly alkaline, relatively favorable for the development of coriander.

The biogenicity of the investigated variants includes the determination of ammonifiers (non-sporing bacteria and bacilli), actinomycetes and micromycetes (Table 3).

Table 3. Quantity and qualitative composition of soil microorganisms (CFU x 10^3 / g abs. dry soil)

Variant	Total micro-flora	Non-spore-forming bacteria	Bacilli	Actinomycetes	Micromycetes	Bacteria assimilating mineral nitrogen	Mineralization coefficient
Control (before placing the trial, no vegetation)	3259.2	2667.2	400	57.6	134.4	4920	1.60
Coriandrum sativum L. (at the end of the growing season)	4102	2953.6	506.8	292	349.6	5936.4	1.72

The results show that the biogenicity of the soils is higher in the variant with vegetation compared to the control (no vegetation). This trend applies to the individual studied groups of microorganisms and, accordingly, to the general microflora. The rate of decomposition of organic matter in soils correlates with the amount of microorganisms.

In both variants, non-spore-forming bacteria take the main share in the composition of the general microflora, followed by bacilli, and the least represented are micromycetes (mold fungi) and actinomycetes (Figures 1 and 2).

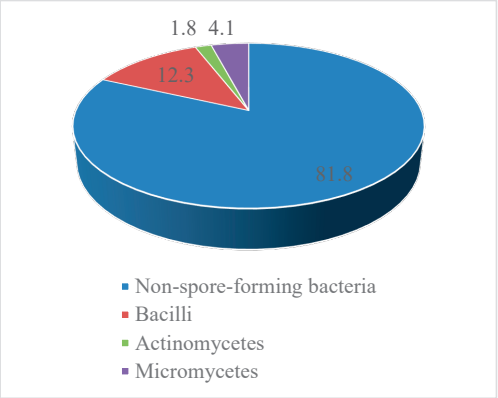

Figure 1. Percentage participation of microorganisms in the composition of the total microflora (control)

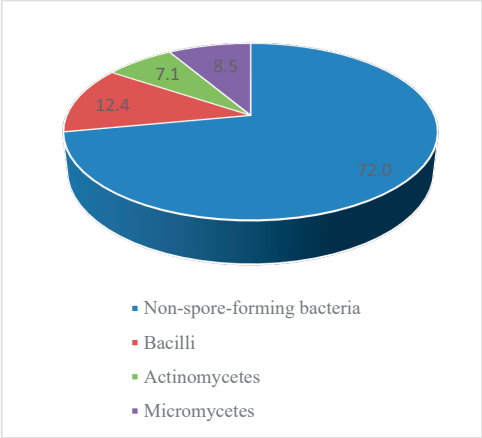

Figure 2. Percentage participation of microorganisms in the composition of the total microflora (*Coriandrum sativum* L.)

The percentage participation of non-spore-forming bacteria in the variant without vegetation is higher, while in the variant with coriander, the amount of this group of microorganisms decreases at the expense of an increase in the amount of actinomycetes and micromycetes. The amount of spore-forming bacteria - bacilli - remains relatively constant in both variants. Non-spore-forming bacteria and bacilli are mainly involved in the initial stages, and actinomycetes and micromycetes in the final stages of decomposition of organic matter.

Catalase is a respiratory enzyme that breaks down H_2O_2 (toxic), which is released when

proteins are broken down. The catalase activity of the tested variants is presented in Figure 3.

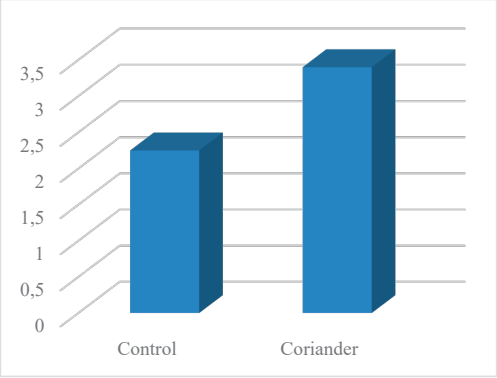

Figure 3. Catalase activity of soil microorganisms (ml O_2/30 min)

The results showed that in the variant with coriander, catalase activity increased 1.5 times, compared to the variant without vegetation. In addition to microbial origin, there is also catalase of plant origin. Catalase values correlate with the amount of soil microorganisms. A number of factors are important for enzyme activity: soil type, soil humidity and temperature, nutrient content, amount and composition of microflora, type of vegetation and others.

Cellulase catalyzes the hydrolysis of cellulose, in which cellulose is initially broken down to cellobiose, which under the action of ß-glucosidase is broken down to glucose. The cellulase activity of the tested variants is presented in Figure 4.

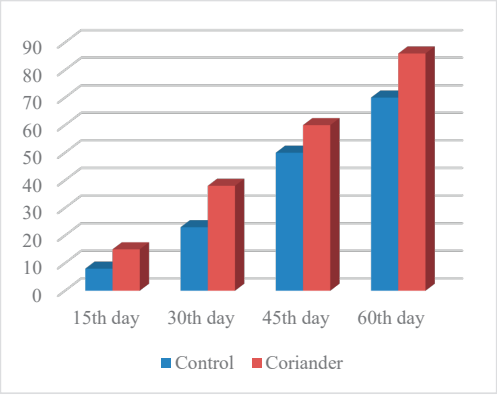

Figure 4. Cellulase activity of soil microorganisms (% degraded area)

Microbiological and enzymatic activity were approached by a number of authors as sensitive soil indicators, including contamination with pathogenic microorganisms (Malcheva et al., 2021; Malcheva et al., 2022, Dilly et al., 2003; Nannipieri et al., 2000; Li et al., 2008; Perucci, 1992; Pascual et al., 1999; García-Gil et al., 2000; Ros et al., 2003; Crecchio et al., 2004; Bastida et al., 2008; Marcote et al., 2001; Malcheva, 2014a, 2014b).

The results were negative for the presence of the tested pathogenic species *E. coli* in both variants, which allowed to test the prepared variant coriander plant extracts for antimicrobial activity (Table 4). Studies on the antioxidant and antimicrobial activity of *C. sativum* have mainly focused on the aerial parts (Kačániová & Ivanišová, 2019).

Table 4. Antimicrobial activity against *Escherichia coli* of the studied coriander extracts

Variant	Sterile area, cm			
	Roots	Leaves	Stems	Whole plant
Decoct	1.3	0.2	0.2	0.2
Tincture	0.9	0.6	0.5	0.9
Medicinal wine	1.4	0.8	1.1	15
Medicinal vinegar	0.7	0.5	0.5	0.8
Medicinal oil	1.5	0.9	1.3	1.5

The sterile zone for coriander root extracts decreases in the following order: medicinal oil > medicinal vinegar > decoction > tincture > medicinal wine. Compared to the root extracts, the sterile zone of the leaf extracts had lower values, indicating that coriander roots had a more effective effect against *E. coli* compared to their leaves. The sterile zone for coriander leaf extracts decreases in the following order: medicinal oil > medicinal vinegar > tincture > medicinal wine > decoction. Compared to root and leaf extracts, the sterile zone of stem extracts was intermediate between the results for root and leaf, indicating that coriander roots had a more effective effect and leaves had a weaker effect against *E.coli* compared to its stems. In medicinal wine, the same effect against the pathogen was found for leaf and stem extracts. The sterile zone for coriander stem extracts decreases in the following order: medicinal oil > medicinal vinegar > tincture = medicinal wine > decoction. Compared to other whole plant

extracts, the sterile zone is comparable to root extracts – the largest retention zone compared to leaf and stem extracts. It is found that the sterile zone in the decoction variant is greater only in the root extracts, in the leaf, stem and whole plant extracts it is the lowest. The sterile zone for coriander whole plant extracts decreases in the following order: medicinal oil > medicinal vinegar > tincture > medicinal wine > decoction. In terms of antimicrobial activity, gram-negative bacteria (*E. coli* and others) are more sensitive to coriander oil than gram-positive bacteria (Saygi et al., 2021; Silva et al., 2011). Similar results against *E. coli* were obtained in the study of antibacterial activity of savory oil (Blažeković et al., 2010).

The nature of the solvent was found to be the main factor in the extraction of antioxidants and bioactive compounds from coriander (Kačániová et al., 2020). Additional conditions - lowering the pH of the medium when using medicinal vinegar and medicinal wine, the inclusion of additional plants - grapes (wine), vinegar (apples, grapes), olives (solvent olive oil for the medicinal oil) also affect the retention zone. In the variants with medicinal vinegar, a general sterile zone is formed around the wells, in the variants with medicinal wine - a bubble halo at a distance of 0.5 cm around each well - probably reactions from the created acidic environment. In the medicated oil variants, the sterile zone increases towards the interior, showing an enhanced effect of combining the root, leaf, stem, whole plant variants and combining the coriander extracts with olive extract (olive oil). As the time of action of the extracts increases (24 h, 48 h, 72 h), the diameter of the sterile zone increases by about 0.1 cm. Essential oil from the plant *Satureja hortensis* L. manifested varied antibacterial activity against *E.coli*, *Salmonella enteritidis*, and *B. subtilis*, depending on the concentration of essential oil used as well as the type of bacteria (Blažeković et al., 2010).

CONCLUSIONS

The obtained results of the agrochemical analyzes indicate that *Coriandrum sativum* does not have a great influence on the dynamics of macroelements in the soil. The macronutrient values obtained were close at the beginning and end of the experiment.

The variants with coriander increased the biogenicity of soil microorganisms, but in general the composition and percentage participation of the studied groups of microorganisms were preserved. In all variants, the main share in the composition of the general microflora is occupied by non-spore-forming bacteria, followed by bacilli, and the least represented are actinomycetes and micromycetes.

The values of the enzymes catalase and cellulase correlate with the amount of microorganisms and also their activity increases in the variant with coriander. A number of factors are important for enzyme activity: soil type, soil humidity and temperature, content of nutritional elements, amount and composition of microflora, type of vegetation and others.

Root and whole plant extracts showed higher antimicrobial activity against *E. coli* compared to leaf and stem extracts. The strongest antimicrobial activity of the plant extracts was found in the medicated oil and medicated vinegar variants and the weakest in the "decoction" variants (except for the root extracts).

The choice of solvent probably affects the diameter of the retention zone. As the exposure time increases, the sterile zone increases.
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